Subterranean Biology 52: 111–133 (2025)
doi: 10.3897/subtbiol.52.142113
https://subtbiol.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

Mixtacandona thessalica, a new species of ostracod (Crustacea, Ostracoda) from a sulfidic cave in central Greece

Giampaolo Rossetti[1], Serban M. Sarbu[2,3], Andrei Ștefan[2,4], Rozalia Motoc[4], Ilaria Mazzini[5]

1 *Department of Chemistry, Life Science and Environmental Sustainability, University of Parma, 43124 Parma, Italy* **2** *"Emil Racoviță" Institute of Speleology, 400006 Cluj Napoca, Romania* **3** *California State University, Department of Biological Sciences, Chico, CA, 95973, USA* **4** *"Grigore Antipa" National Museum of Natural History, 011341 Bucharest, Romania* **5** *CNR - IGAG, Area della Ricerca di Roma 1, 00015 Montelibretti, Rome, Italy*

Corresponding author: Ilaria Mazzini (ilaria.mazzini@cnr.it)

Academic editor: Fabio Stoch | Received 19 November 2024 | Accepted 12 December 2024 | Published 3 June 2025

https://zoobank.org/B8B54667-384D-4498-BE7F-BBA4439AEAFD

Citation: Rossetti G, Sarbu SM, Ștefan A, Motoc R, Mazzini I (2025) *Mixtacandona thessalica*, a new species of ostracod (Crustacea, Ostracoda) from a sulfidic cave in central Greece. Subterranean Biology 52: 111–133. https://doi.org/10.3897/subtbiol.52.142113

Abstract

The genus *Mixtacandona* Klie, 1938 (Crustacea, Ostracoda, Candonidae) includes 21 living non-marine species, all subterranean, with Palearctic distribution. Here we report on *Mixtacandona thessalica* **sp. nov.**, collected in a sulfidic cave in central Greece. It can be considered an extremophile species because of its ability to thrive in an environment with high concentrations of sulfide and reduced chemical compounds. *Mixtacandona thessalica* **sp. nov.** belongs to the species group *laisi–chappuisi*, one of the five groups in which species of the genus are conventionally placed. A detailed morphological description of the valves and the soft parts of the new species is offered. In addition, COI and 28S genetic markers were sequenced. *Mixtacandona thessalica* **sp. nov.** is easily distinguishable from the other species in the genus by its peculiar carapace outline and the marked sexual dimorphism of the posterior margin of valves, as well as by details of chaetotactic characters. The discovery of *Mixtacandona thessalica* **sp. nov.** increases the number of non-marine ostracod species known from Greece, which is still rather scarce compared to other Mediterranean countries due to the scarcity of studies. Ten hypogeal species of living ostracods have already been reported from Greece, six of which are considered endemic, and among them three belong to the genus *Mixtacandona*. It is stressed that a comprehensive review of this genus by combining a thorough morphological approach and molecular techniques, is most needed to assess its phylogenetic relationships within the family Candonidae.

Copyright *Giampaolo Rossetti et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Keywords
Candonidae, COI and 28S sequences, Melissotrypa Cave, morphology, subterranean ostracods, sulfidic environment, taxonomy

Introduction

Ostracods are a class of bivalve crustaceans that have undergone an extraordinary evolutionary radiation and are characterized by an almost continuous fossil record (Horne 2003). Ostracods appeared in marine environments during the late Cambrian (Siveter et al. 2024), and the first invasions of continental waters date back to the Carboniferous (Rodriguez-Lazaro and Ruiz-Muñoz 2012; Iglikowska 2014). Ostracods have been used as model organisms in numerous evolutionary, ecological and biogeographical studies (Martens and Horne 2000).

The extant non-marine ostracods all belong to the order Podocopida G.O. Sars, 1866, in which more than 2300 species in about 270 genera have been described (Meisch et al. 2019). Non-marine ostracods have colonized a wide variety of aquatic and semi-terrestrial ecosystems, including subterranean habitats such as hyporheic zones, alluvial aquifers and caves. A considerable share of the subterranean ostracod species described so far belong to the subfamily Candoninae Kaufmann, 1900, in which three groups of species can be distinguished based on the degree of colonization of the subterranean realm (Danielopol 1980): in the first there are no exclusively subterranean species (stygoxenes, e.g. the genus *Candona* Baird, 1845), the second includes both epigean and subterranean species (e.g. species in the genera *Fabaeformiscandona* Krstić, 1972 and *Pseudocandona* Kaufmann, 1900), while the third consists of subterranean dwellers only (stygobites, e.g. the genera *Phreatocandona* Danielopol, 1973 and *Mixtacandona* Klie, 1938). Accordingly, the degree of morphological and physiological adaptations to subterranean environments also varies, e.g. reduction in body size, anophthalmia, chemo– and mechanoreceptor development, predominantly or exclusively sexual reproduction, and reduction in the number of eggs produced (Moldovan 2018).

Most species of subterranean ostracods are considered endemic, but this estimation is undoubtedly affected by the still limited number of studies that have analyzed these invertebrate faunas.

Freshwater caves (i.e. those that are not marine or anchialine) frequently host ostracods. Martínez (2023) reported that studies conducted in 1577 freshwater caves across the world produced 389 records of non-marine ostracods, totaling 141 species of which 59 were exclusively found in caves. Among this category of subterranean ecosystems, chemosynthesis-based sulfidic caves are particularly interesting due to the peculiar chemical characteristics of their waters and, despite the extremely stressful environmental conditions, the possibility of hosting unexpectedly rich faunas (Engel 2007; Hillebrand-Voiculescu 2018; Pop et al. 2023; De Waele et al. 2024). Regarding ostracods, records from these ecosystems are extremely limited.

Mixtacandona sp. and *Pseudolimnocythere* sp., two new putative species, were found in sulfidic waters of the Frasassi cave system, Italy (Peterson et al. 2013; Sarbu et al. 2022). *Pseudocandona movilaensis* Iepure, Wysocka & Namiotko, 2023 was described from a sulfidic cave in Romania (Iepure et al. 2023). Popa et al. (2019) reported the presence of ostracods belonging to the genus *Mixtacandona* from Melissotrypa cave in central Greece, pointing out close morphological similarities with *M. idrisi* Mazzini & Rossetti, 2017, a species described from a cave near Palermo in Sicily (Mazzini et al. 2017) and possibly occurring as a fossil in north-eastern Spain (Iriarte et al. 2023).

In this paper, a complete morphological description of the valves and soft parts of the *Mixtacandona* ostracods from Melissotrypa Cave is provided, allowing us to describe it as a new species. In addition, the COI and 28S DNA markers were sequenced for comparison with the molecular data available for other congeneric species and to assess the phylogenetic relationships of *Mixtacandona* within the family Candonidae Kaufmann, 1900.

Materials and methods

Study area

Melissotrypa Cave (39°52'40"N, 22°02'57"E) is a hypogenic cave located close to Elassona, in central Greece, at an altitude of 299 m (Vaxevanopoulos 2006). The cave contains a 5 m deep sulfidic lake, and two non-sulfidic lakes in the southern and eastern maze areas (Fig. 1). Temperatures of 16° to 19 °C have been measured in the air and in the lakes, respectively. The rich food base for its abundant and diverse subterranean invertebrate community (Popa et al. 2019) is generated by chemosynthesis *in situ* by sulfur-oxidizing microorganisms that use hydrogen sulfide from the cave water and dioxygen from the cave atmosphere. Numerous specimens of ostracods belonging to the genus *Mixtacandona* Klie, 1938 have been observed in the Sulfidic Lake and in the Gallery Lake. Four additional endemic stygobitic species have been described from Melissotrypa Cave: two gastropods (*Iglica hellenica* Falniowski & Sarbu, 2015 and *Daphniolla magdalenae* Falniowski & Sarbu, 2015), one isopod (*Turcolana lepturoides* Prevorčnik, Konec & Sket, 2016), and one amphipod (*Niphargus gammariformis* Borko, Collette, Brad, Zakšek, Flot, Vaxevanpoulos, Sarbu & Fišer, 2019); two oligochaetes (*Haplotaxis* sp. nov. and *Delaya* sp. nov.), one planarian (*Dendrocoelum* sp. nov.), and one amphipod (*Bogidiella* sp. nov.) are still waiting to be described (Popa et al. 2019).

Ostracod sampling and morphological analysis

Ostracods were collected during two surveys (5 May, 2023 and 29 June, 2024) using a 180 µm hand net and were preserved in 70% ethanol. Sorting and dissections of ostracods were done under a stereomicroscope (Zeiss 47 50 22). Carapaces were measured under a light microscope equipped with a calibrated micrometer ocular. In most cases, intact

Figure 1. Plan of Melissotrypa Cave (modified after Vaxevanopoulos 2006). The location of the cave in the Greek Mainland is marked with a star.

valves could not be preserved due to their extreme fragility. Whenever possible, partially damaged valves or valve fragments were stored dry in micropalaeontological slides. Digital images of carapaces and valves were acquired using scanning electron microscopy (ZEISS EVO MA10 – SEM). The dissected soft parts were mounted in glycerine on microscope glass slides and sealed using nail polish. Line drawings of soft parts were made with the help of a camera lucida-equipped microscope. The chaetotaxy of the soft parts follows Meisch (2000). All the figured specimens are adults.

The specimens examined in this study labeled with the abbreviation GR followed by a number are stored in the first author's ostracod collection at the University of

Parma, Italy. Specimens used for SEM of soft parts are labeled with a four-digit number. Type material is deposited in the crustacean collection of La Specola Museum of Natural History, Zoology Section a Florence, Italy; the number after the acronym MZUF indicates the collection number of the deposited specimens.

Molecular analyses

The specimens used in the molecular analyses were stored in 96% ethanol and kept at -20 °C until further processing (n = 7 specimens of *M. thessalica* sp. nov., and n = 5 specimens of *M. idrisi*). DNA extraction was carried out using the QIAmp® DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer specifications. A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the ZplankF1 (5'-TCTASWAATCATAARGATATTGG-3') and ZplankR1 (5'-TTCAGGRTGRCCRAARAATCA-3') primer pair (Prosser at al. 2013), and a fragment of the nuclear 28S rDNA was amplified using the 28SA (5'-GACCCGTCTTGAAGCACG-3') and 28SB (5'-TCGGAAGGAACCAGCTAC-3') primer pair (Whiting 2002). PCRs were performed in 40 µl volumes containing 1X AccuStart II ToughMix (Quantabio, Beverly MA, USA), 1X Loading Dye, 0.1 µM each primer and DNA template. Thermal cycling for COI amplification consisted of an initial denaturation at 94 °C for 3 minutes followed by 35 cycles of denaturation at 94 °C for 30 seconds, primer annealing at 51 °C for 30 seconds and elongation at 72 °C for 50 seconds. The cycling conditions for 28S were the same, except for the primer annealing temperature, which was 55 °C. Amplification was confirmed on 1.5% (w/v) agarose gels stained with ethidium bromide, and sequencing was performed at a sequencing facility (Macrogen, Amsterdam, The Netherlands).

The resulting chromatograms were visually inspected and edited using Chromas v.2.6.6 (Technelysium Ltd., South Brisbane, Australia) and assembled in CodonCode Aligner v.3.7.1 (CodonCode Corporation, MA, USA). The manually curated sequences were checked against GenBank (Sayers et al. 2022) using BLASTn (Altschul et al. 1990) and the most similar sequences from the Candonidae family were downloaded and included in the analysis. Sequence alignment for the two sets (COI and 28S) were done on the MAFFT web server (https://mafft.cbrc.jp/alignment/server/index.html, accessed on 21 October 2024) using default parameters, phylogenetic reconstruction was performed by Maximum Likelihood on the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/, accessed on 21 October 2024), and the phylogenetic trees were visualized in FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 23 March 2023). The substitution saturation for COI was checked in DAMBE v.7.3.32 (Xia 2017) using the method described in Xia et al. (2003). Species delimitation analysis was done using the distance-based method ASAP (Assemble Species by Automatic Partitioning; Puillandre et al. 2021) and the tree-based method PTP (Poisson Tree Processes; Zhang et al. 2013). Basic sequence statistics and haplotype identity analysis were performed in DnaSP v.6 (Rozas et

al. 2017) and p-distances between species were calculated in MEGA v.7 (Kumar et al. 2016). MOLD v.1.4 was used to identify diagnostic nucleotide combinations (DNCs) in the COI sequence alignment, which can be used to provide formal diagnoses of taxa (Fedosov et al. 2022).

Abbreviations used in the text and figures

Valves. **Cp**: carapace; **dv**: dorsal view; **ev**: external view; **H**: height; **iv**: internal view; **L**: length; **LV**: left valve; **lv**: lateral view; **RV**: right valve; **W**: width.

Soft parts. **A1**: antennule; **A2**: antenna; **T1**: first thoracopod (maxilliped); **T2**: second thoracopod (walking leg); **T3**: third thoracopod (cleaning leg); **CR**: caudal ramus; exo: exopodite on A2; **ya**: aesthetasc on A1; **t1-4** and **z1–3**: setae or male bristles on A2; **Y** and **y1-3**: aesthetascs on A2; **G1-3, GM, Gm**: claws on A2; **f, g** and **h1-3**: setae and claws on T2 and T3; **d1-2** and **dp**: setae on T3; **Sa** and **Sp**: anterior and posterior setae on CR; **Ga** and **Gp**: anterior and posterior claws on CR; **a, b** and **h**: outer, inner and medial lobes of hemipenis.

Results

Taxonomic account

Class Ostracoda Latreille, 1802
Subclass Podocopa G.O. Sars, 1866
Order Podocopida G.O. Sars, 1866
Suborder Cypridocopina Baird, 1845
Superfamily Cypridoidea Baird, 1845
Family Candonidae Kaufmann, 1900
Subfamily Candoninae Kaufmann, 1900
Tribe Candonini Kaufmann, 1900

Genus *Mixtacandona* Klie, 1938

Diagnosis of the genus (after Mazzini et al. 2017). carapace 0.48–0.80 mm long, W < 1/3 L. Calcified inner lamella of valves narrow, marginal pore canals rare. Valve shape in lateral view trapezoidal, triangular or elongated, surface smooth or slightly ornamented. A2 of male with or without male bristles, aesthetasc Y conspicuously long (≥ 60% of first endopodal segment). T1–respiratory plate with three filaments. Male T1–palps (clasping organs) only slightly asymmetrical. T3 four– or five-segmented; protopodite with three setae (d_1, d_2, and d_p), seta f often present, distal segment with one long (h_3) and two very short setae (h_1 and h_2). CR with short S_p. Hemipenis with a finger-shaped outer lobe, M-process absent. Eye pigment absent. Exclusively stygobitic species.

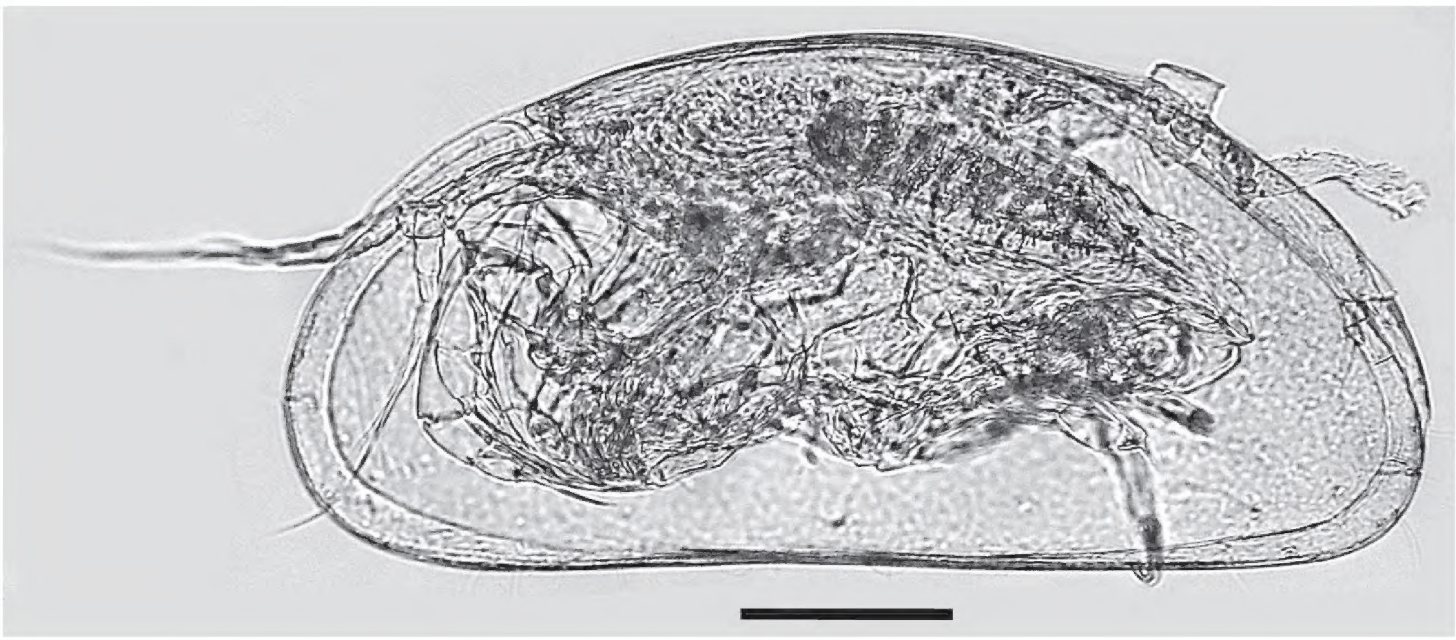

Figure 2. *Mixtacandona thessalica* sp. nov., ♂ (GR975–MZUF693). Optical microscope image of entire body in interior of right valve after removing left valve. Scale bar: 100 μm.

Mixtacandona thessalica Rossetti & Mazzini, sp. nov.

https://zoobank.org/7343C8DE-9834-4C6C-8878-D8539C5AAEB5

Figs 2–7A, B

Note. Authorship of the new species is attributed to G.R. and I.M. and should be cited as "Rossetti and Mazzini" in "Rossetti et al." (ICZN 2000, Recommendation 51E).

Type locality. Sulfidic lake in Melissotrypa Cave, Elassona, Greece, 39.877778°N, 22.049167°E, 299 m a.s.l.

Type material. ***Holotype*:** • adult ♂ (GR972–MZUF691): soft parts dissected in glycerine in a sealed slide, valves stored dry in a micropalaeontological slide (LV damaged). ***Paratypes*:** • one adult ♂ (GR973–MZUF692) with soft parts dissected as the holotype, valves stored dry in a micropalaeontological slide (LV slightly damaged and fragments of RV); • one adult ♂ (GR975–MZUF693) with soft parts dissected as the holotype; • two adult ♀♀ (GR974–MZUF694 and GR985–MZUF695) with soft parts dissected as the holotype; • two adult ♂♂ and two adult ♀♀ stored *in toto* in ethanol (no numbers). All type material was collected by S.M.S. on 5 May, 2023 (GR985–MZUF695) and 29 June, 2024 (GR972–MZUF69, GR973–MZUF692, GR974–MZUF694, GR975–MZUF693) and stored in 96% ethanol.

Other material examined. About 50 specimens from the same samples of the type material, partly used for dissections and/or SEM and the remaining ones preserved in ethanol. The material is stored in the ostracod collection of the first author.

Etymology. The specific name derives from the Latin adjective "*thessalicus*" (conjugated feminine), indicating the origin from Thessaly, the region of Greece where Melissotrypa Cave is located.

Measurements. L of ♂♂ (n = 9): range 520–558 μm, mean ± SD 546.0 ± 12.1 μm; L of ♀♀ (n = 4): range 543–585 μm, mean ± SD 569.5 ± 18.3 μm.

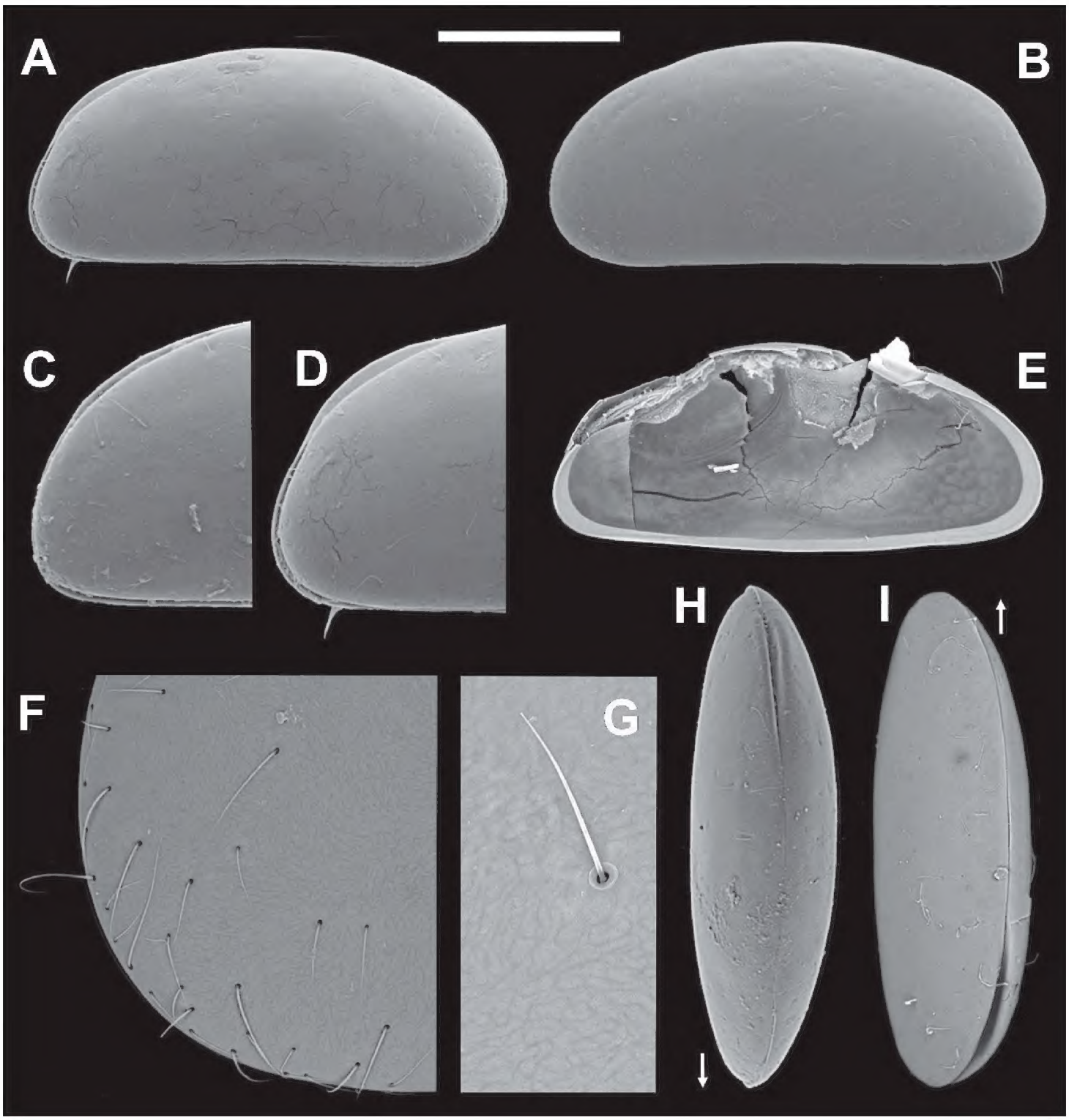

Figure 3. *Mixtacandona thessalica* sp. nov. **A** ♂, Cp right lv (GR976) **B** ♂, Cp left lv (GR977) **C** ♀, Cp right lv, detail posterior part (GR978) **D** ♂, Cp right lv, detail posterior part (GR976) **E** ♂, LV iv partly damaged (0060) **F** ♂, detail of reticulate pattern of external valve surface (0024) **G** ♂, rimmed pore canal with its seta (0024) **H** ♀, Cp oblique dv (GR979) **I** ♂, Cp oblique dv (0024). Scale bar: 200 µm (**A, B, E, H, I**); 150 µm (**C, D**); 50 µm (**F**); 20 µm (**G**).

Diagnosis. Small-medium sized *Mixtacandona*, belonging to the *laisi–chappuisi* species group (see Discussion). Females slightly larger than males, but with some overlap in lengths. Cp with an elongated, lateral outline (H/L ≅ 0.45 in both sexes) and narrow in dorsal view (W < 1/3 of L). Valve surface smooth, covered with sparse setae. LV slightly overlapping RV on all sides, more markedly in the postero-dorsal corner, especially in males. Ventral margin straight, dorsal margin gently arched, greatest height at middle length. Posterior margin rounded in females, straight in males. Aesthetasc Y on A2 approximately as long as the first endopodal segment. A2 with

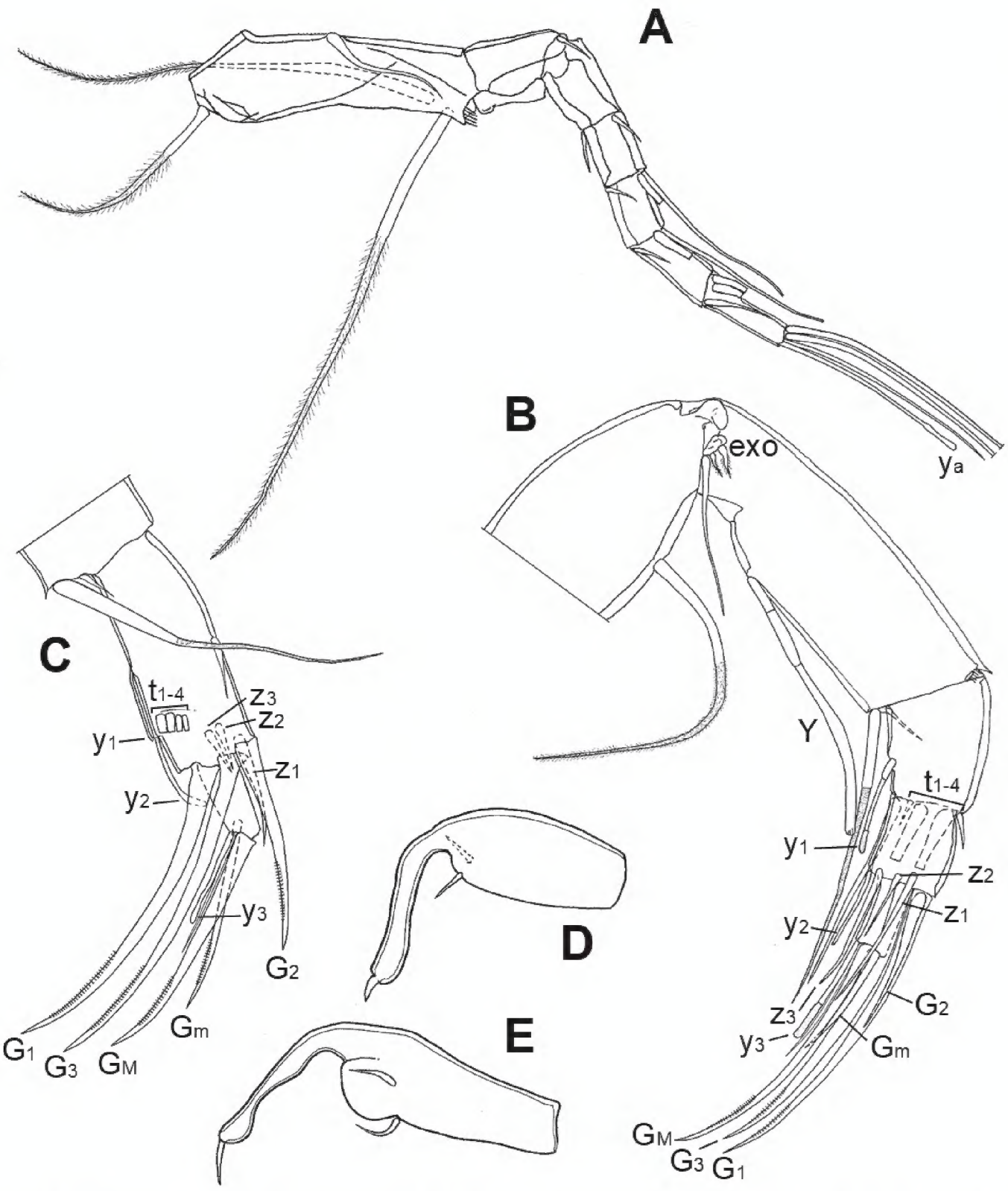

Figure 4. *Mixtacandona thessalica* sp. nov. **A** ♂, A1 (first segment partially twisted) (GR972–MZUF691) **B** ♂, A2 (GR972–MZUF691) **C** ♀, terminal part of A2 (GR985–MZUF695) **D** ♂, left T1–palp (GR972–MZUF691) **E** ♂, right T1–palp (GR972–MZUF691). Scale bar: 100 μm.

second endopodal segment subdivided in males and undivided in females, setae t2 and t3 transformed into bristles in males. Seta f on T2 present. T3 with a three-segmented endopodite (second and third endopodal segments partially fused), seta h2 longer than last endopodal segment, seta h3 c. as long as the endopodite.

Description. Carapace and valves. Male Cp in lateral view with elongate, subtrapezoidal shape (Fig. 3A, B). Greatest H around mid-length. Ventral margin straight. RV: Dorsal margin gently arched, anteriorly slightly sloping more steeply than posteriorly. Greatest length just below mid-height. Anterior margin rounded.

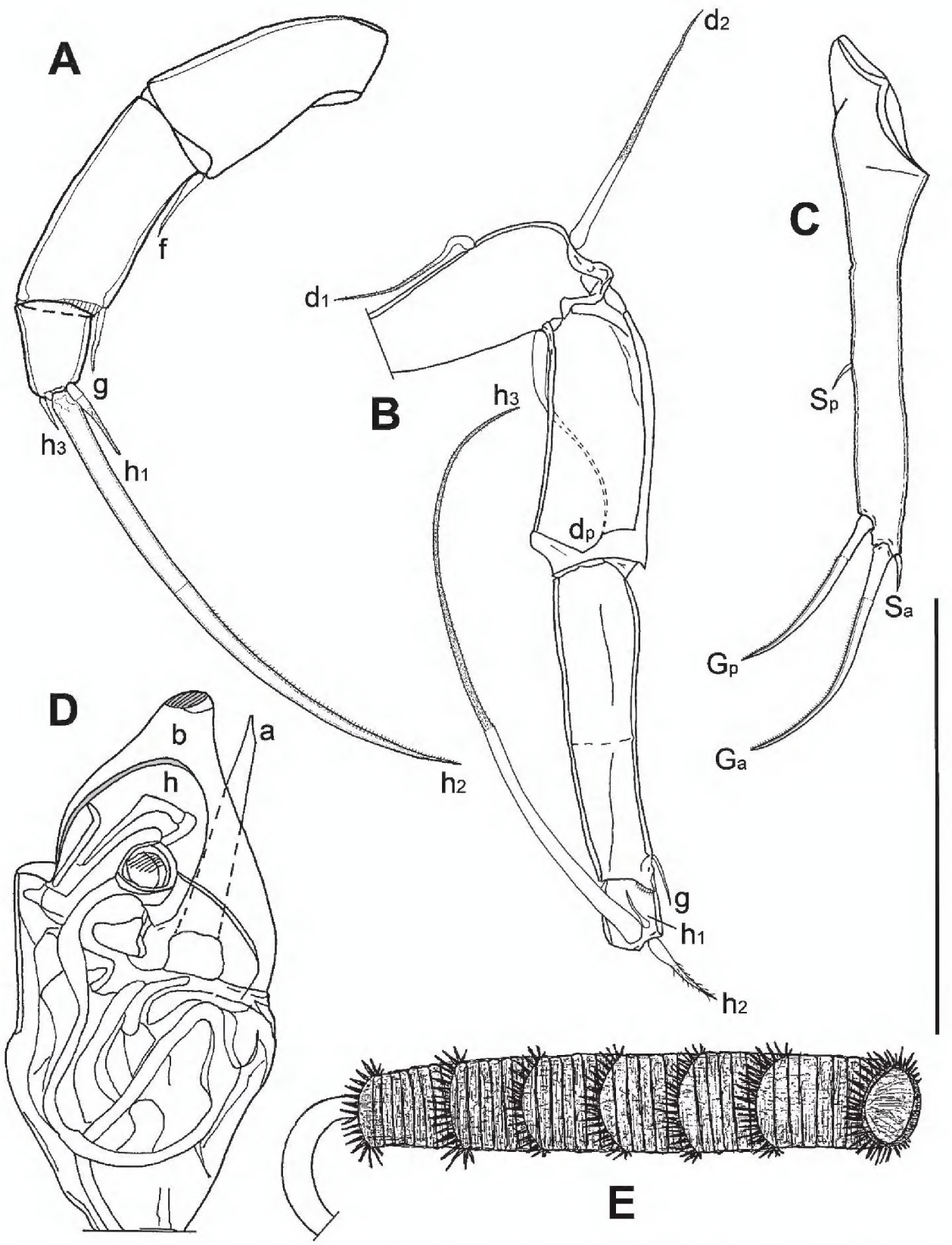

Figure 5. *Mixtacandona thessalica* sp. nov. **A** ♂, T2, distal part of endopodite (GR972–MZUF691) **B** ♂, T3 (GR972–MZUF691) **C** ♂, CR (GR972–MZUF691) **D** ♂, hemipenis (GR972–MZUF691) **E** ♂, Zenker organ (GR973–MZUF692). Scale bar: 100 μm (**A–C, E**); 80 μm (**D**).

Posterior margin bluntly more pointed than anterior one (Fig. 3A). LV: general outline similar to RV but with a subtle posterior dorsal protuberance that overlaps RV, forming an obtuse angle with the dorsal margin. The characteristic posterior protuberance is more evident in males (Fig. 3D) than in females (Fig. 3C). Surface ornamentation consists of a delicate pattern similar to a vascular pattern (Fig. 3F, G). Simple pore canals with a lip and sensory seta (Fig. 3G). LV overlaps RV at both ends (Fig. 3 H, I). Marginal pore canals short, simple and scattered. Central muscle scar arrangement as characteristic for the genus.

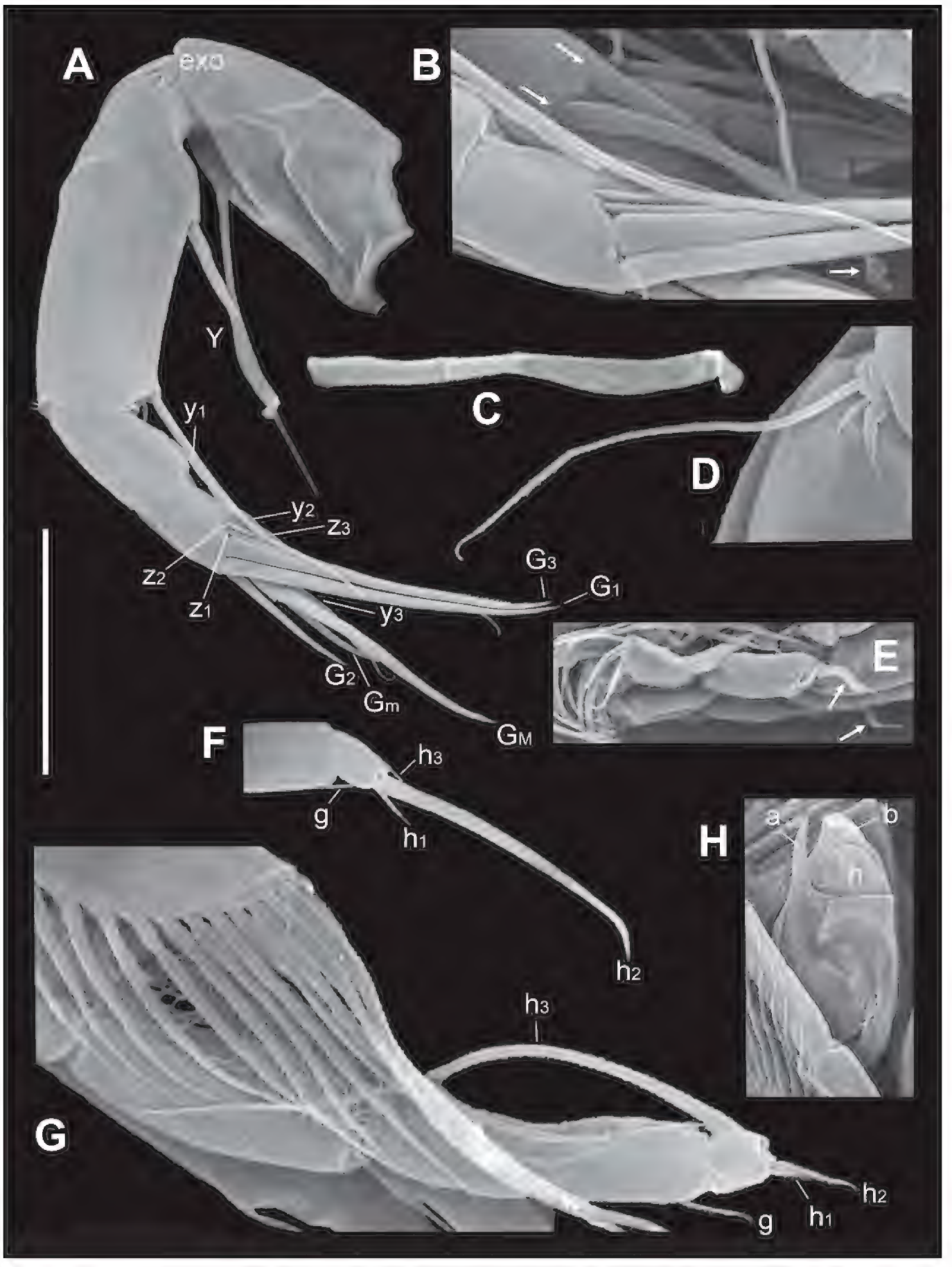

Figure 6. *Mixtacandona thessalica* sp. nov. **A** ♂, A2 (0061) **B** ♂, A2, detail male bristles (arrows) (0061) **C** ♂, A2, detail aesthetasc Y (0061) **D** ♀, A2, detail exopodite (0036) **E** ♂, T1–palps (arrows) (0061) **F** ♀, terminal part of T2 (0036) **G** ♀, branchial plate of maxillula (foreground) and T3 (0036) **H** ♂, hemipenis (0061). Scale bar: 100 µm (**A, B, E–H**); 25 µm (**C, D**).

Soft parts. A1 (Fig. 4A): first segment with two long dorsal setae and two shorter setae on the ventral margin; second segment with a short ventral seta apically; third segment with short apical setae, one ventral and one dorsal; fourth and fifth segments with a short dorsal seta and two long, unequal ventral setae greatly exceeding tip of last segment;

sixth segment with a dorsal setae reaching c. 2/3 of the next segment and two very long ventral setae; seventh (terminal) segment bearing two very long setae and a shorter one, the latter slightly longer than aesthetasc ya. A2 ♂ (Figs 4B, 6A–C): protopodite with a long ventral seta; exopodite with one long seta and two tiny, unequal setae; endopodite four-segmented (second segment subdivided); first endopodal segment with aesthetasc Y placed at c. 1/3 of the ventral margin and as long as the segment, three-segmented (distal part widened), and two ventro-apical setae, one largely exceeding the last segment of endopodite and the other very tiny; second endopodal segment bearing a sub-apical aesthetasc y_1 and an apical seta ventrally, and on the internal side setae t_{1-4}, of which the two median transformed into bristles; third endopodal segment with sub-equal apical claws (G_1 and G_3), more dorsally another claw (G_2) slightly shorter than half the length of the previous ones, three setae (z_{1-3}) in sub-apical position, and a ventro-apical aesthetasc (y_2); fourth segment with two claws, one (G_m) about 70% the length of the other (G_M), and an apical aesthetasc (y_3) with its companion seta ventrally. A2 ♀ (Figs 4C, 6D): endopodite three-segmented (second segment undivided); second endopodal segment with short seta inserted at the half of the dorsal margin, unequal setae $t_{1–4}$, setae z_2 and z_3 about 2/5 as long as seta z_1, aesthetascs y_1 and y_2 respectively at mid and sub-apical position along the ventral margin, apical claw G_2 c. 3/5 the length of G_1 and G_3; third endopodal segment distally with claw G_m about 3/4 the length of G_M, and aesthetasc y_3 with its companion seta. T1 ♂ (Figs 4D, E, 6E): endopodite palps slightly asymmetrical, left one slightly more bumped and sinuous than right one. T2 (Figs 5A; 6F): endopodite four-segmented, second and third segments with apical short setae (f and g); fourth segment with a stout, terminal claw h_2 slightly longer than the three distal endopodal segments, flanked by setae h_1 and h_3, the latter very tiny and c. 1/3 of h_1. T3 (Figs 5B, 6G): protopodite with setae d_p and d_2 subequal and approximately as long as the next segment, seta d_1 shorter than previous ones; endopodite three-segmented (second segment partially subdivided); first endopodal segment without setae, second segment with a tiny sub-apical seta (g); third segment with three apical setae, the first (h_1) very small, the second (h_2) a little longer than the distal endopod segment, the third (h_3) slightly shorter than the endopodite. CR (Fig. 5C): seta S_p inserted at about 2/3 of the posterior margin; seta S_a strongly reduced, claw G_p c. 3/4 G_a. Hemipenis (Figs 5D, 6H): median part of inner margin straight, lobe a thin and pointed distally, lobe b roughly triangular with the apical part folded and thickened, lobe h thumb-shaped with chitinous distal margin. Zenker organ (Fig. 5E): thin and with 5+2 spinous whirls. Eye absent. Other appendages as typical for the genus *Mixtacandona*.

Differential morphological diagnosis. *Mixtacandona thessalica* sp. nov. is easily distinguished from the other species of the genus having a smooth, elongated, and dorsally curved carapace by its peculiar valve outline and the marked sexual dimorphism in the posterior margin (Fig. 7). It can be also differentiated by the combination of chaetotaxic characters details reported above. COI sequences confirm the distinctness of this species (see below), although the available data allow comparison with a small number of congeneric species.

Molecular analysis. Seven sequences of *M. thessalica* sp. nov. and four sequences of *M. idrisi* were obtained for COI, with a length of 663 bp. The sequence quality

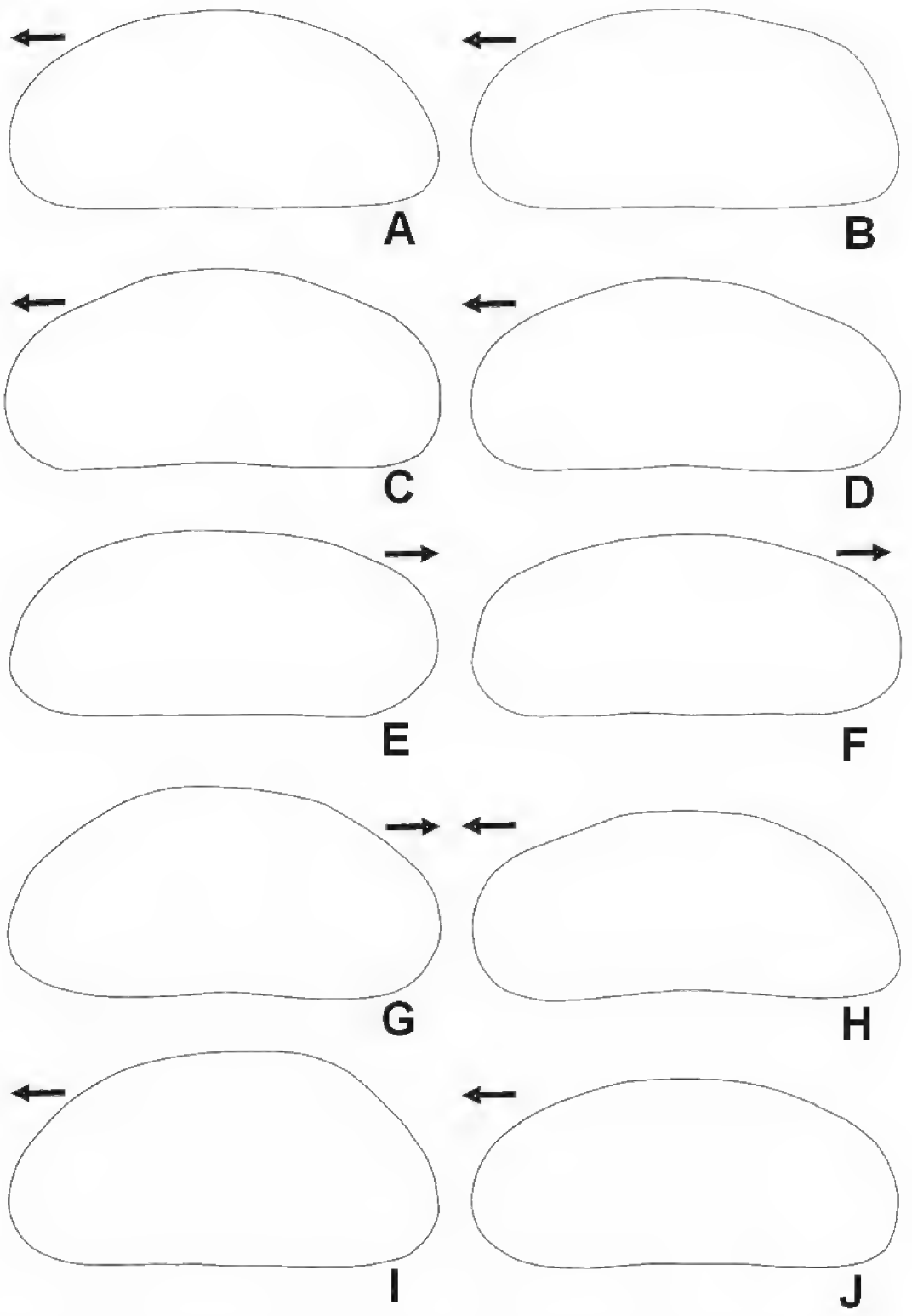

Figure 7. Comparison of outlines of species with curved dorsal valve margins assigned to the *laisi–chappuisi* group in the genus *Mixtacandona* (all figures redrawn to the same length) **A** *Mixtacandona thessalica* sp. nov., ♀, Cp left lv **B** *Mixtacandona thessalica* sp. nov., ♂, Cp left lv **C** *Mixtacandona idrisi*, ♂, Cp left lv **D** *Mixtacandona idrisi*, ♀, Cp left lv **E** *Mixtacandona pseudocrenulata*, ♂, LV iv **F** *Mixtacandona pseudocrenulata*, ♀, LV iv **G** *Mixtacandona chappuisi*, ♂, LV iv **H** *Mixtacandona chappuisi*, ♀, LV ev **I** *Mixtacandona laisi*, ♀, LV ev **J** *Mixtacandona transleithanica*, ♀, LV ev. **C, D** from Mazzini et al. 2017; **E, F** from Schäfer 1945; **G, H** from Klie 1943; **I** from Klie 1938; **J** from Löffler 1960.

and the presence of indels or early stop codons were checked. Four haplotypes were present in the seven isolates of *M. thessalica* sp. nov.: Hap1 (*M. thessalica* sp. nov. isolate MEL1), Hap2 (*M. thessalica* sp. nov. isolate MEL2, *M. thessalica* sp. nov. isolate MEL5, *M. thessalica* sp. nov. isolate MEL6, *M. thessalica* sp. nov. isolate MEL7), Hap3 (*M. thessalica* sp. nov. isolate MEL3), and Hap4 (*M. thessalica* sp. nov. isolate MEL4). Only one haplotype was present in the isolates of *Mixtacandona idrisi*: Hap1 (*M. idrisi* isolate IT1,

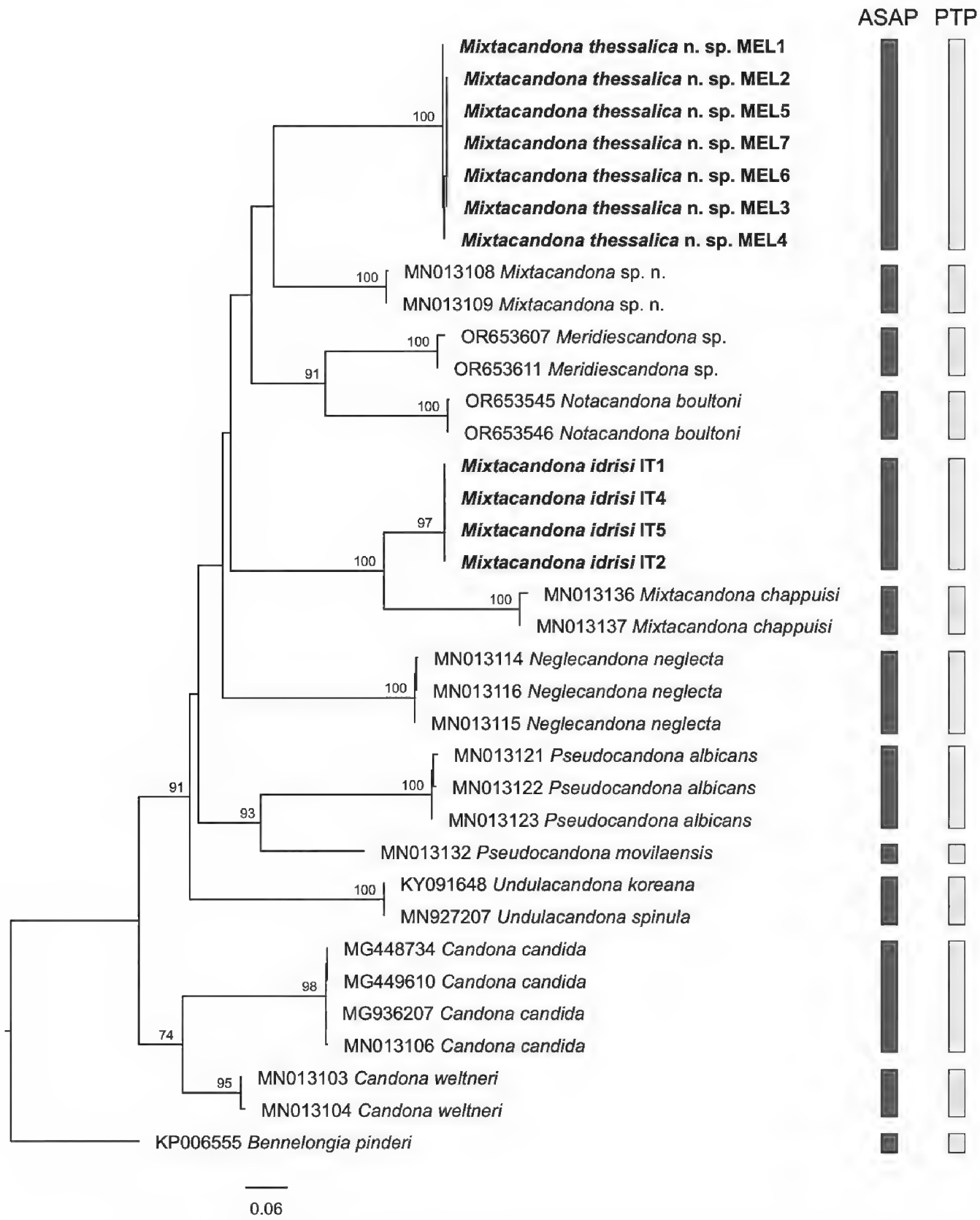

Figure 8. Maximum Likelihood phylogenetic tree of available species in the family Candonidae using COI sequences. Species delimitation results by ASAP (dark gray) and PTP (light gray) are also shown. *Bennelongia pinderi* Martens et al., 2015 was used as outgroup. The tree is drawn to scale, with branch lengths measured in the number of nucleotide substitutions per site. The scalebar represents the number of substitutions per unit of branch length. Bootstrap values larger than 70% after 1000 replicates are shown on the branches.

M. idrisi isolate IT2, *M. idrisi* isolate IT4, *M. idrisi* isolate IT5). GenBank sequences were included in the analyses and the COI alignment was trimmed to the length of the shortest GenBank sequence, i.e., 515 bp. GenBank accession numbers for our sequences and the downloaded sequences are provided in the Suppl. material 1. Maximum Likelihood phylogeny and p-distances (0.178, $p < 0.05$) indicate that the closest species to

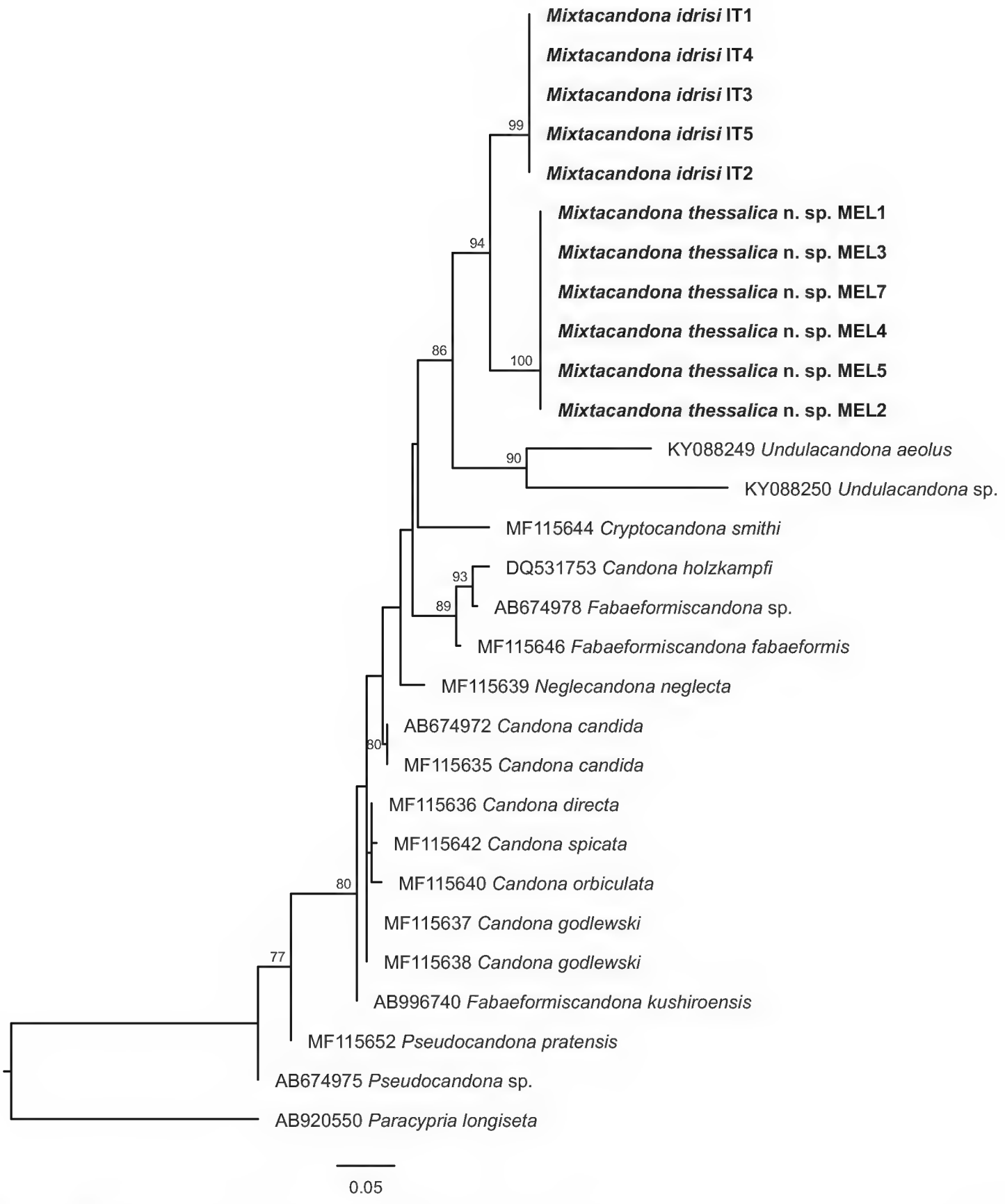

Figure 9. Maximum Likelihood phylogenetic tree for 28S. *Paracypria longiseta* Hiruta & Kakui, 2017 was used as outgroup.

M. thessalica sp. nov. is *Mixtacandona* sp. n. (GenBank accession numbers MN013108 and MN013109) from the Frasassi caves in Central Italy. Both ASAP and PTP clearly distinguish *M. thessalica* sp. nov. as a distinct species. The occurrence of two other genera reported from Australia, *Notacandona* and *Meridiescandona*, within the clade of four *Mixtacandona* species is an unusual result, indicating the possibility of polyphyly in the *Mixtacandona* genus (Fig. 8). Polyphyly was also described in other morphogenera of freshwater Candonidae such as *Candona*, *Fabaeformiscandona*, and *Pseudocandona*

(Karanovic and Sitnikova 2017; Wysocka et al. 2019). Six sequences of *M. thessalica* sp. nov. were obtained for the 28S marker and all shared the same haplotype. Five sequences of *M. idrisi* were also obtained and all shared the same haplotype. The sequence alignment was trimmed to the shortest GenBank sequence (279 bp) and the ML phylogeny and p-distances (0.065, $p < 0.05$) indicate that *M. thessalica* sp. nov. is closest to *M. idrisi*. The absence of 28S sequences in GenBank made it impossible to compare these species to other representatives of the genus *Mixtacandona* (Fig. 9).

Differential molecular diagnosis. (COI): 'T' at site 32, 'C' at site 40, 'G' at site 171 on the 515 bp-long alignment (corresponding to sites 116, 124 and 171, respectively, on the 663 bp full length sequence).

Distribution. The species is only known from its type locality.

Remark. Males and females were equally represented in the analyzed samples.

Discussion

All the 22 extant recognised species of the genus *Mixtacandona* including *M. thessalica* sp. nov. have a western-Palearctic distribution (Meisch et al. 2019). Actually, the number of species is expected to be considerably higher, considering the difficulties involved in sampling subterranean aquatic ecosystems and the taxonomic expertise required to describe species that are often very small and whose identification relies on minute morphological features. As reported by Wysocka et al. (2019), the subfamily Candoninae is one of the most taxonomically difficult lineages of non-marine Ostracoda. In the literature, there are numerous reports of living and fossil *Mixtacandona*, some even of putative new species, but with largely insufficient or completely missing descriptions.

Mixtacandona thessalica sp. nov. can be considered an extremophile species, based on the chemical characteristics of the water in Melissotrypa Cave, with high concentrations of sulfide and reduced compounds. It can be assumed that its presence in this habitat is the result of adaptive processes that occurred over long timescales. Information on the ecology of this species is still lacking. It is possible that, as observed for *Mixtacandona* sp. in the Frasassi cave system, this species occupies oxygenated water layers close to the sulfidic chemocline, where sulfur-oxidizing bacteria abound and constitute an almost unlimited trophic supply (Peterson et al. 2013). The sex ratio in the population of *M. thessalica* sp. nov. was close to 1:1. In many stygobitic crustacean species, a balanced sex ratio or even a predominance of males may result in a reproductive advantage in environments characterized by substantial thermal stability, where females display asynchronous mating behavior (Tabilio Di Camillo et al. 2023).

The discovery of *Mixtacandona thessalica* sp. nov. allows us to expand the list of species known from the ostracod fauna of Greece, which, compared to other Mediterranean countries, is still rather limited due to the scarcity of studies (Danielopol 1981; Karanovic 2003; Marrone et al. 2019a, 2019b). There are 10 hypogeal species of living ostracods reported from Greece so far, of which six are currently considered endemic,

Table 1. List of extant subterranean species of ostracods known for Greece (Danielopol 1981; Karanovic 2003). The asterisk indicates species endemic to Greece. A putative new species of *Mixtacandona* (reported as *Trapezicandona* sp. nov.?) from a cave in Greece (Valavani et al. 2024) is not listed here because not yet fully illustrated and formally described.

Species	Family	Habitat	Locality
**Candonopsis thienemani* Schäfer, 1945	Candonidae	well	Armenio
**Candonopsis trichota* Schäfer, 1945	Candonidae	well	Armenio
Kliella hyaloderma Schäfer, 1945	Kliellidae	well	Armenio
Kovalevskiella bulgarica (Danielopol, 1970)	Limnocytheridae	well	Igoumenitsa
**Kovalevskiella dani* Karanovic, 2003	Limnocytheridae	well	Lesbos
Kovalevskiella rudjakovi (Danielopol, 1969)	Limnocytheridae	well	Lesbos
**Mixtacandona peliaca* (Schäfer, 1945)	Candonidae	well	Armenio
**Mixtacandona pseudocrenulata* (Schäfer, 1945)	Candonidae	well	Larissa
"	"	well	Euboea
**Mixtacandona thessalica* sp. nov.	Candonidae	cave	Elassona
**Nannokliella dictyoconcha* Schäfer, 1945	Kliellidae	well	Armenio
*[§]*Pseudolimnocythere hartmanni* Danielopol, 1979	Loxoconchidae	well	Euboea

[§] Aiello et al. (2020) reported *P. hartmanni* as a fossil from the Gulf of Salerno, Italy. If confirmed, this record would invalidate the endemic status for this species (see Rossetti et al. 2022).

and among them three belong to the genus *Mixtacandona* (Table 1). The latter are all included in the *laisi–chappuisi* species group, namely the one with rectangular or trapezoidal outline in lateral view, small to middle sized and with smooth valve surface among the five groups established by Danielopol (1978) for this genus. The different groups can be separated based on size, shape and ornamentation of the carapace (for a discussion on their validity for taxonomic or zoogeographic purposes, see Mazzini et al. 2017). It is therefore plausible to speculate that this geographical area potentially played a crucial role as a speciation hotspot for this group of *Mixtacandona* species, representatives of which have existed in Europe since at least the Miocene (Danielopol 1972, 1980; Mazzini et al. 2017). It can also be seen from Table 1 that the only known cave ostracod is *Mixtacandona thessalica* sp. nov. This further confirms how research on subterranean biodiversity is still limited in Greece, a country that has a complex geological landscape in which the Hellenic Speleological Society has recorded > 10000 caves (Trimmis 2015).

As the carapace is the first point of contact with the external environment, many ostracod species have evolved a highly plastic carapace, whereas the soft parts remain much more conservative (Karanovic et al. 2019). For example, it has been suggested that triangular and trapezoidal shapes are the result of homoplastic adaptations in species dwelling in subterranean environments (Iepure et al. 2023). In the case of *Mixtacandona*, which consists exclusively of stygobitic species, very different carapace morphologies are observed, but without a strict relation to habitat type, e.g. alluvial aquifers or caves. At least one species, *M. botosaneanui* Danielopol, 1973, has been found in both environments (Danielopol 1982; Danielopol and Hartmann 1986).

The taxonomy of the Candoninae remains unclear and generic relationships ambiguous due to a number of homoplasies (Wysocka et al. 2019). This also applies to *Mixtacandona*. In species for which detailed descriptions of the soft parts are available, a marked variability is found in the chaetotaxy. Such differences in appendage morphology are probably sufficient for the establishment of new genera. A comprehensive review of the genus, including the re-examination of all available types, is therefore advisable.

To solve complex questions of taxonomy, phylogeny, but also biogeographical models related to the genus *Mixtacandona* and other ostracod taxa, it is deemed necessary to combine a purely morphological approach with molecular techniques. Such an approach seems appropriate to resolve the phylogenetic inconsistencies in the Candoninae. Wysocka et al. (2019) mention the decoupling between morphological species assignments and genetic data between other non-marine ostracods as well. So, probably, more sequenced markers, with varying degrees of variability and more sequenced ostracod species could shed more light and help disentangle these complex phylogenies. But, for now, the almost complete absence of publicly available sequences for species of *Mixtacandona* makes it impossible to assess their phylogenetic relationships and more molecular studies are clearly needed. This is more evident in the case of the 28S marker, which is completely absent from sequence databases for *Mixtacandona*, but the short overlap (279 bp) in the sequence alignment between the sequences from this study was still enough to separate *M. thessalica* sp. nov. and *M. idrisi*. The genera *Candona* and *Fabaeformiscandona* appear polyphyletic on 28S as well (Fig. 9), but we could not assess the potential 28S polyphyly of *Mixtacandona*, since there are no available sequences to compare them to. Molecular methods have made it possible to document the occurrence of a considerable number of cryptic species of non-marine ostracods, which are indistinguishable from each other through even extremely thorough morphological analysis (Kilikowska et al. 2024). Several approaches using DNA sequencing data have been proved to have a great potential in improving our knowledge on freshwater meiofaunal biodiversity, especially in habitats difficult to sample, such as caves (Schenk and Fontaneto 2020). At the same time, detailed information on the morphology, intraspecific variability and sexual dimorphism of ostracod valves is essential to provide diagnostic characters that can be used in palaeontological research.

Acknowledgments

We thank the members of the speleological teams from Greece and Romania who helped us reach the subterranean sites and assisted with the field work. Luisa Dainelli (University of Pisa) kindly helped with the shipment of specimens used in this study. Dr Matteo Paciucci (CNR-IGAG) is thanked for patiently taking the SEM photos of the specimens. This research was partially funded by Biodiversa+, the European Biodiversity Partnership under the 2021–2022 BiodivProtect joint call for research proposals, co-funded by the European Commission (GA N°101052342) and with the funding organizations Ministry of Universities and Research (Italy), Agencia Estatal

de Investigación—Fundación Biodiversidad (Spain), Fundo Regional para a Ciência e Tecnologia (Portugal), Suomen Akatemia—Ministry of the Environment (Finland), Belgian Science Policy (Belgium), Agence Nationale de la Recherche (France), Deutsche Forschungsgemeinschaft e.V.—BMBF-VDI/VDE INNOVATION + TECHNIK GMBH (Germany), Schweizerischer Nationalfonds zur Forderung der Wissenschaftlichen Forschung (Switzerland), Fonds zur Förderung der Wissenschaftlichen Forschung (Germany), Ministry of Education, Science and Sport (Slovenia), and the Executive Agency for Higher Education, Research, Development and Innovation Funding (Romania). Two anonymous reviewers are acknowledged for their careful and constructive comments, which greatly improved the quality of this manuscript.

References

Aiello G, Amato V, Barra D, Caporaso L, Caruso T, Giaccio B, Parisi R, Rossi A (2020) Late Quaternary benthic foraminiferal and ostracod response to palaeoenvironmental changes in a Mediterranean coastal area, Port of Salerno, Tyrrhenian Sea. Regional Studies in Marine Science 40: 101498. https://doi.org/10.1016/j.rsma.2020.101498

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215: 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Borko Š, Collette M, Brad T, Zakšek V, Flot JF, Vaxevanopoulos M, Sarbu, MM, Fišer C (2019) Amphipods in a Greek cave with sulphidic and non-sulphidic water: phylogenetically clustered and ecologically divergent. Systematics and Biodiversity 17(6): 558–572. https://doi.org/10.1080/14772000.2019.1670273

Danielopol D (1972) On the probable belonging of some fossil *Candona* to the group *Mixta* Klie 1938 (Ostracoda, Podocopida). In: Pokorny V (Ed.) Ostracoda, Symposium, I.P.U., Proceedings 23rd International Geological Congress, Warszawa, 123–126.

Danielopol DL (1978) Ostracodes hypogés du Sud de la France. 1. *Mixtacandona juberthiae*, International Journal of Speleology 9: 235–249. https://doi.org/10.5038/1827-806X.9.3.3

Danielopol DL (1980) An essay to assess the age of the freshwater interstitial ostracods of Europe. Bijdragen tot de Dierkunde 50(2): 243–291. https://doi.org/10.1163/26660644-05002001

Danielopol DL (1981) Distribution of Ostracods in the Groundwater of the North Western Coast of Euboea (Greece). International Journal of Speleology 11(1): 91–103. https://doi.org/10.5038/1827-806X.11.1.10

Danielopol DL (1982) Three groundwater Candoninae (Ostracoda) from Romania. International Journal of Speleology 12: 83–102 https://doi.org/10.5038/1827-806X.12.1.9

Danielopol DL, Hartmann G (1986) Ostracoda. Part I: Stigobiont Ostracoda from inland subterranean waters. In: Botosaneanu L (Ed.) Stygofauna Mundi, EJ Brill, Leiden, 266–279. https://doi.org/10.1163/9789004631977_032

De Waele J, D'Angeli IM, Audra P, Plan L, Palmer AN (2024) Sulfuric acid caves of the world: A review. Earth-Science Reviews 250: 104693. https://doi.org/10.1016/j.earscirev.2024.104693

Engel AS (2007) Observations on the biodiversity of sulfidic karst habitats. Journal of Cave and Karst Studies 69(1): 187–206.

Falniowski A, Sarbu S (2015) Two new Truncatelloidea species from Melissotrypa Cave in Greece (Caenogastropoda). ZooKeys 530: 1–14. https://doi.org/10.3897/zookeys.530.6137

Fedosov A, Achaz G, Gontchar A, Puillandre N (2022) MOLD, a novel software to compile accurate and reliable DNA diagnoses for taxonomic descriptions. Molecular Ecology Resources 22: 2038–2053. https://doi.org/10.1111/1755-0998.13590

Hillebrand-Voiculescu A (2018) Researches in sulphide-based ecosystems. In: Moldovan OT, Kováč Ľ, Halse S (Eds) Cave Ecology. Ecological Studies, vol 235. Springer, Cham, Switzerland, 351–368. https://doi.org/10.1007/978-3-319-98852-8_16

Horne DJ (2003) Key events in the ecological radiation of the Ostracoda. The Paleontological Society Papers 9: 181–202. https://doi.org/10.1017/S1089332600002205

Iepure S, Wysocka A, Sarbu SM, Kijowska M, Namiotko T (2023) A new extremophile ostracod crustacean from the Movile Cave sulfidic chemoautotrophic ecosystem in Romania. Scientific Reports 13(1): 6112. https://doi.org/10.1038/s41598-023-32573-w

Iglikowska A (2014) Stranded: The conquest of fresh water by marine ostracods. Paleontological Research 18(3): 125–133. https://doi.org/10.2517/2014PR014

Iriarte E, Revelles J, Finsinger W, Mesquita-Joanes F, Rodrigo MA, Burjachs F, Expósito I, Marti Molist J, Planagumà Ll, Alcalde G, Saña M (2023) Youngest Iberian Holocene volcanic eruptions and paleoenvironmental evolution of a barrier-paleolake in the Garrotxa Volcanic Field (NE Spain). The Holocene 33(8): 939–959. https://doi.org/10.1177/09596836231169989

Karanovic I (2003) The genus *Kovalenskiella* Klein, 1963 from the ground waters of Greece, with description of *Kovalenskiella dani*, spec. nov., and a key to world recent species (Crustacea, Ostracoda, Limnocytheridae). Spixiana 26(3): 227–242.

Karanovic I, Huyen, PTM, Brandão SN (2019) Ostracod shell plasticity across longitudinal and bathymetric ranges. Deep Sea Research Part I: Oceanographic Research Papers 143: 115–126. https://doi.org/10.1016/j.dsr.2018.11.005

Karanovic I, Sitnikova TY (2017) Morphological and molecular diversity of Lake Baikal candonid ostracods, with description of a new genus. ZooKeys 684: 19–56. https://doi.org/10.3897/zookeys.684.13249

Kilikowska A, Schön I, Wysocka A, Pieri V, Kaczmarczyk-Ziemba A, Prais K, Martens K, Namiotko T (2024) Multiple genetic species in a halophilic non-marine ostracod (Crustacea). Hydrobiologia 851(10): 2447–2467.

Klie W (1938) Ostracoden aus dem Grundwasser der oberrheinischen Tiefebene. Archiv für Naturgeschichte (n. ser.) 7: 1–28.

Klie W (1943) Ostracoden aus dem Grundwasser der Umgebung von Kolozvar. Fragmenta Faunistica Hungarica 6: 35–41.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Löffler H (1960) Die Entomostrakenfauna der Ziehbrunnen und einiger Quellen des nördlichen Burgenlandes (No. 24). Burgenländisches Landesmuseum, 32 pp.

Marrone F, Alfonso G, Stoch F, Pieri V, Alonso M, Dretakis M, Naselli-Flores L (2019a) An account on the non-malacostracan crustacean fauna from the inland waters of Crete, Greece, with the synonymization of *Arctodiaptomus piliger* Brehm, 1955 with *Arctodiaptomus alpinus* (Imhof, 1885) (Copepoda: Calanoida). Limnetica 38(1): 167–187. https://doi.org/10.23818/limn.38.01

Marrone F, Arculeo M, Georgiadis C, & Stoch F (2019b) On the non-malacostracan crustaceans (Crustacea: Branchiopoda, Copepoda, Ostracoda) from the inland waters of Fthiotida (Greece). Biogeographia-The Journal of Integrative Biogeography 34: 87–99. https://doi.org/10.21426/B634043868

Martens K, Horne DJ (2000) Preface: Ostracoda and the four pillars of evolutionary wisdom. Hydrobiologia 419: 7–11. https://doi.org/10.1023/A:1003994820756

Martínez A (2023) Cave Meiofauna-Models for Ecology and Evolution. In: Giere O, Schratzberger M (eds) New Horizons in Meiobenthos Research: Profiles, Patterns and Potentials, Cham: Springer International Publishing, 329–361. https://doi.org/10.1007/978-3-031-21622-0_10

Mazzini I, Marrone F, Arculeo M, Rossetti G (2017) Revision of Recent and fossil *Mixtacandona* Klie 1938 (Ostracoda, Candonidae) from Italy, with description of a new species. Zootaxa 4221(3): 323–340. https://doi.org/10.11646/zootaxa.4221.3.3

Meisch C (2000) Freshwater Ostracoda of Western and Central Europe. In: Schwoerbel J, Zwick P (Eds) Süßwasserfauna von Mitteleuropa 8/3, Spektrum Akademischer Verlag, Heidelberg, Berlin, 522 pp.

Meisch C, Smith RJ, Martens K (2019) A subjective global checklist of the extant non-marine Ostracoda (Crustacea). European Journal of Taxonomy 492: 1–135. https://doi.org/10.5852/ejt.2019.492

Moldovan OT (2018) An overview on the aquatic cave fauna. In: Moldovan OT, Kováč Ľ, Halse S (Eds) Cave ecology, vol 235. Springer, Cham, Switzerland, 173–194. https://doi.org/10.1007/978-3-319-98852-8_8

Peterson DE, Danielopol DL, Finger K, Iepure S, Mariani S, Montanari A, Namiotko T (2013) Reconnaissance of ostracode assemblages in the Frasassi Caves, the adjacent sulfidic spring, and the Sentino River in the northeastern Apennines (Marche region, Italy). Journal of Cave and Karst Studies 75(1): 11–27. https://doi.org/10.4311/2011PA0230

Pop MM, Di Lorenzo T, Iepure S (2023) Living on the edge - An overview of invertebrates from groundwater habitats prone to extreme environmental conditions. Frontiers in Ecology and Evolution 10: 1054841. https://doi.org/10.3389/fevo.2022.1054841

Popa I, Brad T, Vaxevanopoulos M, Giurginca A, Baba SC, Iepure S, Plăiaşu R, Sarbu S (2019) Rich and diverse subterranean invertebrate communities inhabiting Melissotrypa cave in Central Greece. Travaux de l'Institut de Spéologie "Émile Racovitza" 58: 65–78.

Prevorčnik S, Konec M, Sket B (2016) The first *Turcolana* Argano & Pesce, 1980 (Isopoda: Cirolanidae) from the Greek mainland. Zootaxa 4170(1): 125–136. http://doi.org/10.11646/zootaxa.4170.1.6

Prosser S, Martínez-Arce A, Elías-Gutiérrez M (2013) A new set of primers for COI amplification from freshwater microcrustaceans. Molecular Ecology Resources 13: 1151–1155. https://doi.org/10.1111/1755-0998.12132

Puillandre N, Brouillet S, Achaz G (2021) ASAP: assemble species by automatic partitioning. Molecular Ecology Resources 21: 609–620. https://doi.org/10.1111/1755-0998.13281

Rodriguez-Lazaro J, Ruiz-Muñoz F (2012) A general introduction to ostracods: morphology, distribution, fossil record and applications. In: Horne DJ, Holmes J, Rodriguez-Lazaro J, Viehberg FA (Eds) Ostracoda as Proxies for Quaternary Climate Change. Elsevier, Amsterdam, 1–14. https://doi.org/10.1016/B978-0-444-53636-5.00001-9

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins S, Sánchez-Gracia A (2017) DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Molecular Biology and Evolution 34: 3299–3302. https://doi.org/10.1093/molbev/msx248

Sarbu SM, Brad T, Chauveau C, Flot JF, Galdenzi S, Galassi DMP, Gentile G, Iepure, S, Jones DJ, Martin P, Montanari A, Stoch F (2022) Biodiversity in the sulfidic sections of the Frasassi Caves, Italy. Proceedings of the 2nd International Electronic Conference on Diversity (IECD 2022), 1–6.

Sayers EW, Bolton EE, Brister JR, Canese K, Chan J, Comeau DC, Connor R, Funk K, Kelly C, Kim S, Madej T, Marchler-Bauer A, Lanczycki C, Lathrop S, Lu Z, Thibaud-Nissen F, Murphy T, Phan L, Skripchenko Y, Tse T, Wang J, Williams R, Trawick BW, Pruitt KD, Sherr ST (2022) Database resources of the national center for biotechnology information. Nucleic Acids Research 50: D20–D26. https://doi.org/10.1093/nar/gkab1112

Schäfer HW (1945) Grundwasser-Ostracoden aus Griechenland. Archiv für Hydrobiologie 40(4): 847–866.

Schenk J, Fontaneto D (2020) Biodiversity analyses in freshwater meiofauna through DNA sequence data. Hydrobiologia 847(12): 2597–2611. https://doi.org/10.1007/s10750-019-04067-2

Siveter DJ, Briggs DE, Siveter DJ, Sutton MD (2024) Preserved appendages in a Silurian binodicope: implications for the evolutionary history of ostracod crustaceans. Biology Letters 20(5): 20240097. https://doi.org/10.1098/rsbl.2024.0097

Tabilio Di Camillo A, Galassi DMP, Fiasca B, Di Cicco M, Galmarini E, Vaccarelli I, Di Lorenzo T (2023) Variation in copepod morphological and life history traits along a vertical gradient of freshwater habitats. Environments 10(12): 199. https://doi.org/10.3390/environments10120199

Trimmis KP (2015) Hidden treasures in forgotten archives: exploring the archaeology of Greek caves into the archives and the bulletin of the Hellenic Speleological Society. Chronika 5: 32–41.

Valavani D, Papadopoulou P, Frenzel P, Alivernini M, Tsoni M, Groumpou M, Tsourou T, Kolomaznik S, Kreuzheck M, Iliopoulos G (2024) Palaeoenvironmental insights from cave ostracods In Greece: the Cave of the Lakes, Kastria, Greece. Naturalista Siciliano S. IV, 48: 119–120. https://doi.org/10.5281/zenodo.13762368

Vaxevanopoulos M (2006) Tectonic conditions in the speleogenetic process of Melissotrypa cave in Kefalovriso of Elassona (Central Greece). MSc Thesis, School of Geology, Aristotle University of Thessaloniki, Greece, 105 pp. [in Greek]

Whiting MF (2002). Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. Zoologica Scripta 31: 93–104. https://doi.org/10.1046/j.0300-3256.2001.00095.x

Wysocka A, Kilikowska A, Mori N, Iepure S, Kijowska M, Namiotko T (2019) Monophyletic status of European morphogenera of the subfamily Candoninae Kaufmann, 1900 (Ostracoda: Candonidae) in relation to their mtDNA phylogenies. The Journal of Crustacean Biology 39(5): 567–573. https://doi.org/10.1093/jcbiol/ruz041

Xia X (2017) DAMBE6: New Tools for Microbial Genomics, Phylogenetics, and Molecular Evolution. Journal of Heredity 108: 431–437. https://doi.org/10.1093/jhered/esx033

Xia X, Xie Z, Salemi M, Chen L, Wang Y (2003) An index of substitution saturation and its application. Molecular Phylogenetics and Evolution 26: 1–7. https://doi.org/10.1016/s1055–7903(02)00326-3

Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29(22): 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

Supplementary material 1

Ostracod samples

Authors: Andrei Ștefan

Data type: xlsx

Explanation note: List of GenBank accession numbers for our sequences and the downloaded sequences.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/subtbiol.52.142113.suppl1